Automated Detection of Cancerous Cells Using the CellDetect® Technology

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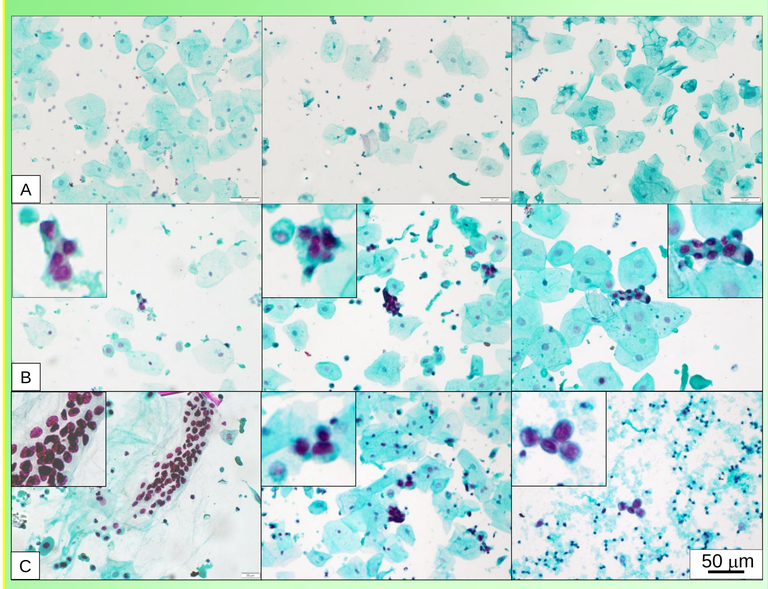
Abstract

Objectives: CellDetect® is unique histochemical-stain enabling color discrimination between normal cells and a variety of neoplastic cells/tissues, including cervix, bladder, colon and circulating tumor cells. Using this technology, normal cells are colored blue/green, while neoplastic cells are stained in red. This tinctorial difference coincides with clear morphological visualization properties.
The growth of digital methods in pathology is accelerating and digital images can be used for a variety of applications in cytology. The purpose of this study was to show feasibility to digitally analyze urine smears stained with the CellDetect® stain.
Methods: 142 images of 37 cytological smears stained by the CellDetect® stain were used. Cases included 13 high grade urothelial carcinoma, 11 low grades and 13 normal cases. Smears were photographed and the images were submitted to a digital analysis.
Results: Few tens of variables were extracted from the analysis and tested for their ability to separate between negative and positive smears. The resultant algorithm could separate well between the two populations, with AUC ranging between 99-99.6%.
Conclusion: This study establishes definite feasibility to accurately diagnose smears stained by CellDetect® by digital analysis of color and morphology.

Introduction

CellDetect® is an innovative histochemical staining platform that combines color and morphology to discriminate between normal and neoplastic cells (see panel below). This feature enhances the accuracy of the detection of bladder tumors in urine smears of patients monitored for the recurrence of the disease[1,2], with sensitivity of 84% and specificity of 83%. The objective of this feasibility study was to evaluate the ability of a dual color and morphology-based image analysis to differentiate between benign and cancer cells.



Photomicrographs of urine smears stained with CellDetect®. (A) urine of normal patients, (B) urine of patients with low grade tumors, (C) urine of patients with high grade tumors. The majority of the cells are normal, epithelial cells that are stained in green. In images B-C a few foci of reddish-purple cancer cells are seen (enlarged in boxes). Magnification: x40

References:
1. Davis et al. *The Journal of Urology* 2014; 192:1628-1632
2. Shtabsky et al. Abstract OFP-03 at the European Congress of Pathology 2015

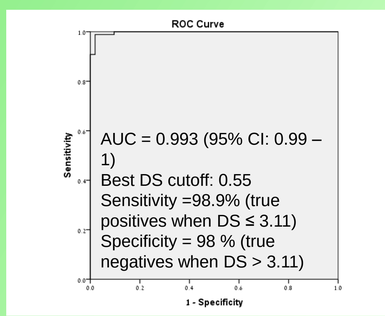
Methods

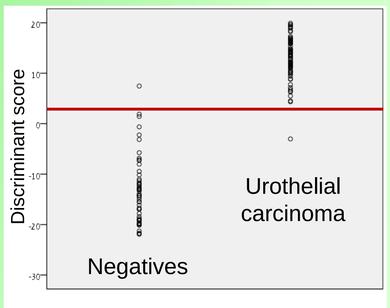
Urine samples were processed to cytospin smears and stained with the CellDetect® stain according to manufacturer's instructions (Zetiq Technologies Ltd.). Case distribution was: 13 high grade urothelial carcinoma, 11 low grades and 13 normal cases. Stained smears were scanned with a bright field BX51 microscope (Olympus) and 142 images containing the cells were captured with DP25 camera (Olympus).
Nuclear morphometry was done with the aid of the Image Pro-Plus software (MediaCybernetics, MA, USA) as follows: after removing artefacts, the color threshold of the nuclei was established and the nuclei were automatically measured by the program. When nuclei overlapped, their contours were semi-automatically segmented and measured. The variables that were extracted from the morphometric analysis included nuclear size descriptors (e.g. area, perimeter, a variety of diameters), nuclear shape descriptors (e.g. contour irregularity, ellipticity, fractal dimension), nuclear texture descriptors (e.g. heterogeneity, margination, clumpiness, standard deviation of pixel gray values) and nuclear staining descriptors (e.g. red, green and blue channels gray values, ranging between 0 to 255 arbitrary units of gray, with low values indicated darker staining).
Comparison of the nuclear morphometry descriptors between the diagnostic groups (negative vs. tumor, negative vs. low grade or negative vs. high grade tumors) was done by univariate analysis followed by a multivariate discriminant regression analysis applied in a Wald forward method. The multivariate algorithm was able to control for confounding variable and then to single out true independent predictors of the diagnostic category. Subsequently, using the regression coefficients of the independent predictors that have been obtained by the multivariate model (constant, slopes), discriminant scores (DS) were calculated, able to predict each one of the diagnostic categories. Best cutoff points (highest sensitivity and specificity) were found in these discriminant scores, using a receiver characteristic curve (ROC) analysis. Two tailed p values of 0.05 or less were considered to be statistically significant.
To compare the performance of the algorithm to that of human eye, all images were also viewed by an expert cytology who was blinded to patient's true diagnosis.

Results

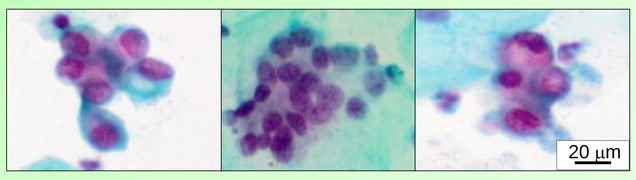
Excellent separation between normal and urothelial cancer images using color and morphology parameters

ROC analysis of the DS to differentiate between negatives versus urothelial carcinoma

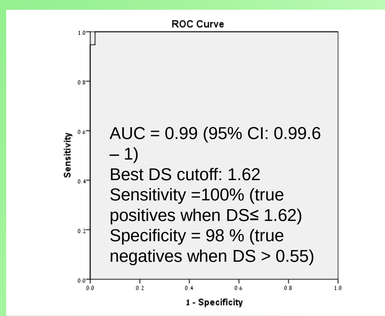


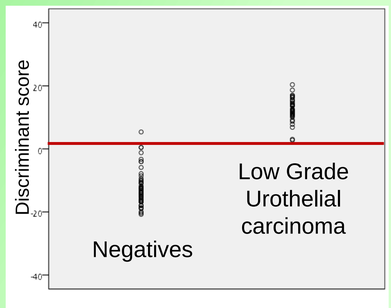


Urine smears stained with CellDetect®, urothelial carcinoma (adopted from [2]).

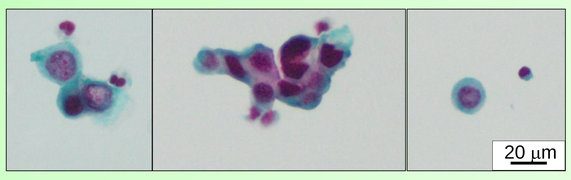


ROC analysis of the DS to differentiate between negatives versus Low Grade cases

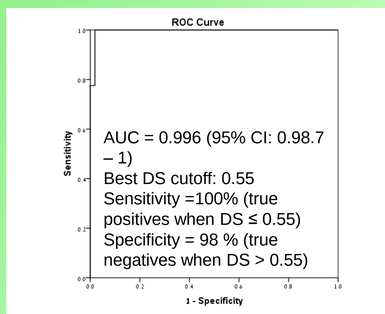


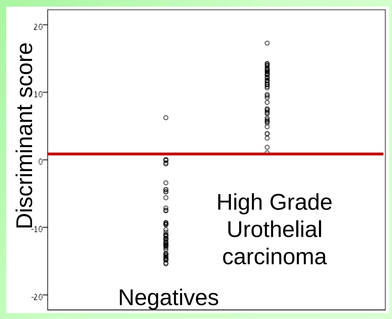


Urine smears stained with CellDetect®, urothelial carcinoma, low grade cells.



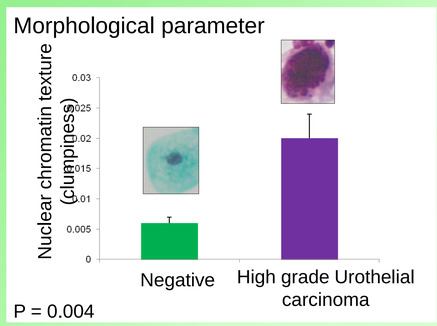
ROC analysis of the DS to differentiate between negatives versus High Grade cases

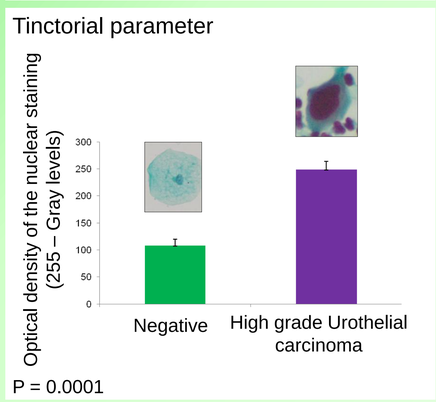




Urine smears stained with CellDetect®, urothelial carcinoma, high grade cells.

Examples for univariate analysis - comparison of single morphological or tinctorial parameters





Human-eye analysis of the images results in similar performance to that of the algorithm

		Gold Standard		
		Urothelial carcinoma	Normal	Total
CellDetect®	Positive	78	0	78
	Negative	9	55	64
	Total	87	55	142

Sensitivity	Specificity	NPV	PPV
90%	100%	100%	86%

Kappa coefficient of agreement = 0.87, 95% CI 0.788 – 0.912

Conclusions

- ✓ The CellDetect® stain enables an accurate diagnosis of urothelial carcinoma by digital algorithm combining color and morphology, as well as by human eye
- ✓ The developed digital algorithm resulted in an excellent separation between normal subjects and urothelial cancer patients, regardless cancer grade (AUC ranges between 99-99.6%)
- ✓ The results provide confidence that automation/semi automation of CellDetect® analysis is feasible, which will allow to combine the high accuracy of the stain and the benefits of digital pathology